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**Multifunctional T-cell Analyses to Study Response and Progression in Adoptive Cell Transfer Immunotherapy.**

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**Public Summary:**

This manuscript describes the application of newly developed nanodiagnostic assays to analyze the function of immune cells used in adoptive cell transfer for patients with melanoma. The results of this study suggest that there is a need to maintain immune cell function long term, providing further support for the need to use stem cells to regenerate a cancer-fighting immune system.

**Scientific Abstract:**

Adoptive cell transfer (ACT) of genetically engineered T cells expressing cancer-specific T-cell receptors (TCR) is a promising cancer treatment. Here, we investigate the in vivo functional activity and dynamics of the transferred cells by analyzing samples from 3 representative patients with melanoma enrolled in a clinical trial of ACT with TCR transgenic T cells targeted against the melanosomal antigen MART-1. The analyses included evaluating 19 secreted proteins from individual cells from phenotypically defined T-cell subpopulations, as well as the enumeration of T cells with TCR antigen specificity for 36 melanoma antigens. These analyses revealed the coordinated functional dynamics of the adoptively transferred, as well as endogenous, T cells, and the importance of highly functional T cells in dominating the antitumor immune response. This study highlights the need to develop approaches to maintaining antitumor T-cell functionality with the aim of increasing the long-term efficacy of TCR-engineered ACT immunotherapy.

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